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IL-13 Can Substitute for IL-4 in the Generation of Dendritic Cells for the Induction of Cytotoxic T Lymphocytes and Gene Therapy

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Summary: Immunization with tumor-associated antigen pulsed dendritic cells (DC) has been shown to elicit both protective and therapeutic antitumor immunity in a variety of animal models and is currently being investigated for the treatment of cancer patients in clinical trials. In this study we show that DC can be generated from peripheral blood mononuclear cells of healthy donors as well as breast and melanoma cancer patients using granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-13 (IL-13) and that these DC have many of the same characteristics as DC differentiated using GM-CSF and IL-4. The DC generated in GM-CSF and IL-13 are $CD14^-$ and express high levels of the cell surface markers CD86, HLA-DR, and CD58, as do DC generated in GM-CSF and IL-4. The purity and yield of both DC populations are not significantly different. Furthermore, both populations of DC are effective at presentation of alloantigen as determined in a mixed lymphocyte response, and both are able to process and present soluble tetanus toxoid antigen to $CD4^+$ T cells. Because we are interested in the generation of DC for antigen-specific cytotoxic T lymphocyte (CTL) generation, we compared the ability of peptide-pulsed DC differentiated in GM-CSF and IL-4 versus GM-CSF and IL-13 for the generation of influenza and MART-1 specific CTL. Both populations of DC induced $CD3^+CD8^+CD4^-$ and $CD56^-$ CTL, which could lyse the appropriate targets in an antigen-specific manner. Finally, both GM-CSF and IL-4 DC and GM-CSF and IL-13 DC yielded similar β galactosidase expression levels after transduction with recombinant adenovirus containing the *LacZ* gene. These results suggest that DC generated in GM-CSF and IL-13 may be useful for immunotherapy and gene therapy protocols. **Key Words:** Tumor immunotherapy—Cytokine—Dendritic cell—Adenovirus.

Successful antigen-specific immunotherapy will require targeting an effective antigen presentation system. Dendritic cells (DC) are professional antigen-presenting cells that provide all the signals needed for $CD4^+$ and $CD8^+$ T-cell activation. They express high levels of major histocompatibility complex (MHC) and costimulatory molecules, produce cytokines, and migrate to the lymphoid organs (1,2). They have been shown to stimulate both a naive and memory T-cell response in vitro (3,4). Furthermore, recent studies indicate that DC are effective at inducing protection from, and eradication of, established tumors in vivo in murine models (5–14). In these studies, DC are either pulsed with tumor antigen peptides, loaded with protein, or infected with recombinant adenovirus expressing tumor antigen.

Historically, the use of DC as immunotherapeutic agents in humans has been difficult because of the low frequency and difficulty of purification of DC. With improved isolation methods (15,16) in which DC are differentiated from progenitors using granulocyte-macrophage colony-stimulating factor (GM-CSF) and

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interleukin-4 (IL-4), DC can now be isolated in sufficient quantity for therapeutic use. As seen for mature DC, these GM-CSF and IL-4 differentiated DC have typical dendritic morphology and express high levels of MHC class I and II, adhesion molecules, and costimulatory molecules. Functionally, DC cultured in GM-CSF and IL-4 are effective at stimulating a mixed lymphocyte response (MLR), presenting tetanus toxoid antigen to T cells (15,16), and inducing antigen-specific cytotoxic T lymphocytes (CTL) in vitro (17) and in vivo (8,9).

During DC culture in GM-CSF and IL-4, the IL-4 is thought to be necessary to suppress the monocyte differentiation potential of a GM-CSF responsive bipotential progenitor (15). IL-4 has been shown to inhibit human macrophage colony formation (18), decrease CD14 expression, and increase accessory potential (19). IL-13 is a cytokine that shares a number of structural characteristics with IL-4 including related intron/exon gene sequence and ~30% amino acid sequence homology. Functionally, the two cytokines are also similar. Both are produced by activated T cells and both act on macrophages to upregulate MHC class II expression (20,21), induce differentiation, and suppress the production of inflammatory cytokines (22). Because IL-13 shares many properties with IL-4, we were interested in determining whether IL-13 can substitute for IL-4 in the generation of DC that could be used for immunotherapy. In this study, we show that DC generated using GM-CSF and IL-13 share many of the same properties as those generated in GM-CSF and IL-4.

MATERIALS AND METHODS

HLA-A2$^+$ Lymphocytes

Buffy coats from healthy volunteers were obtained from the Stanford University Blood Bank or the American Red Cross. After giving informed consent, patients with breast cancer or melanoma underwent leukapheresis according to approved Duke University Medical Center, University Southern California, or Alta Bates Investigational Review Board protocols. For the antigen-specific CTL induction studies, cells were phenotyped for the MHC class I HLA-A2 allele by flow cytometry using monoclonal antibody BB7.2 (23). Peripheral blood mononuclear cells (PBMC) were isolated from both buffy coats and leukapheresis samples by Ficoll-Hypaque density gradient centrifugation.

Cell Lines

The T2 cell line [processing defective cell line that expresses empty A2 molecules until stabilized by the addition of peptide (24)] was purchased from the American Type Culture Collection (ATCC; Rockville, MD, U.S.A.) and was maintained in Dulbecco's Modified Eagle Medium) supplemented with 10% fetal calf serum (FCS) (HyClone Laboratories; Logan, UT, U.S.A.), 2 m*M* L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

DC Isolation

DC were isolated using a modification of Romani et al. (15). Briefly, 1.5×10^8 PBMC were allowed to adhere to T-150 culture flasks for 2 h at 37°C in RPMI-10% FCS. After incubation, the nonadherent cells were removed and the adherent cells were cultured in 25 ml of RPMI-10% FCS medium containing 800 U/ml GM-CSF (Biosource International; Camarillo, CA, U.S.A.) [1×10^7 U/mg] and either 100 ng/ml IL-4 (Genzyme) or 5 ng/ml IL-13 (R&D Systems, Minneapolis, MN, U.S.A.). These concentrations were determined in previous experiments. After 6–7 days of culture, the differentiated DC were harvested and used for assays as described below.

Peptides

The HLA-A2 restricted influenza A matrix [GILGFVFTL (25)] and MART-1 [AAGIGILTV (26)] peptides were purchased from Endocrine Technologies Inc. (Newark, CA, U.S.A.). The lyophilized peptides were dissolved in DMSO at a concentration of 10 μg/ml, and stored at –20°C. For peptide stripping, DC were washed once in cold 0.9% NaCl with 1% bovine serum albumin (BSA) solution, resuspended at 1×10^7 cells/ml in stripping buffer [0.13 *M* L-ascorbic acid, 0.06 *M* sodium phosphate monobasic (pH 3.0), 1% BSA, 3 μg/ml β2-microglobulin (Scripps Laboratories.; San Diego, CA, U.S.A.), 10 μg/ml peptide] and incubated for 2 min on ice. The cells were then neutralized with 5 vol of cold neutralizing buffer [0.15 *M* sodium phosphate monobasic (pH 7.5), 1% BSA, 3 μg/ml β2-microglobulin, 10 μg/ml peptide] and spun at 1,500 r/min for 5 min. Finally, the cells were resuspended in peptide solution [phosphate-buffered saline (PBS)-CMF (calcium magnesium free), 1% BSA, 30 μg/ml DNAase, 3 μg/ml β2-microglobulin, and 40 μg/ml peptide] and incubated for 4 h at room temperature. After incubation, the cells were irradiated (3,000 rad) and washed before being used for stimulation.

CTL Generation

CD8+ responders for peptide-specific CTL were generated by adhering PBMC on Applied Immune Sciences

(Santa Clara, CA, U.S.A.) CD8 T25 MicroCELLector flasks. CD8 captured cells were stimulated with irradiated DC loaded with peptides (flu or MART as indicated) at a stimulator-to-responder (S:R) ratio of 1:3. These cells were cultured in RPMI-10% FCS containing 10 ng/ml IL-7 (R&D Systems). At days 10–12, the lymphocytes were restimulated with DC pulsed with peptide (1:5 S:R ratio). Responders were restimulated weekly to a total of three to four restimulations at S:R ratios ranging between 1:5 and 1:15. As controls, CD8 captured cells were also stimulated with unpulsed DC + IL-7 (10 ng/ml) or IL-7 only (10 ng/ml) (our previous studies indicate that these controls give background levels of cytotoxicity) or PHA/IL-2 [2 μg/ml PHA (phytohemagglutinin) (Wellcome Diagnostics; Dartford, England) and 500 U/ml IL-2 (Cetus Corp., Emeryville, CA, U.S.A.)].

Cytotoxicity Analysis

HLA-A2 restricted antigen recognition by CTL was assessed by a standard 4-h ^{51}Cr release cytotoxicity assay. Recognition of flu or MART peptide by CTL was assessed using T2 cells preincubated for 2–4 h with peptide at 40 μg/ml. Target cells were resuspended at 1×10^6 cell/ml, plated in six-well plates (2 ml/well), and labeled overnight with 100 μCi ^{51}Cr at 37°C. After overnight incubation, targets were washed three times and mixed with effector cells at varying effector-to-target ratios in U-bottom microtiter plates. After a 4-h incubation, supernatants were harvested, and the amount of ^{51}Cr released was measured with the liquid scintillation counter 1205 Beta plate (Wallac Oy; Turku, Finland). Percent specific cytotoxicity was calculated as follows: [(cpm of test sample – cpm of spontaneous ^{51}Cr release)/(cpm of maximal ^{51}Cr release – cpm of spontaneous ^{51}Cr release)] × 100.

Cell Staining and Flow Cytometry

Cells ($3–5 \times 10^5$) were incubated with the corresponding antibody for 25 min at 4°C. The cells were then washed and fixed with 1% paraformaldehyde solution. Three-color flow cytometric analysis was performed with LYSIS software on FACScan (Becton Dickinson, San Jose, CA, U.S.A.). Data from 10,000 cells were collected and analyzed. Donor PBMC were stained with anti-HLA-A2 antibody derived from BB7.2 hybridoma clone (23). DC were phenotyped with antibody to the following markers: CD3, HLA-DR, CD14, CD16, CD19, CD54, CD58 (Becton Dickinson), and CD86 (Pharmingen, San Diego, CA, U.S.A.). The CTLs were phenotyped with antibody against CD3, CD4, CD8, and CD56 (Becton Dickinson).

MLR

DC cultured with GM-CSF and IL-4 or GM-CSF and IL-13 generated from the same donor were irradiated (3,000 rad) and added at various dilutions to 2×10^5 purified allogeneic T cells in U-bottom 96-well plates (Costar, Cambridge, MA, U.S.A.). T cells were purified from PBMC of different healthy donors using a commercially available T-cell enrichment column (R&D Systems). The cells were cultured for 5 days at 37°C (5% CO_2) and then pulsed overnight with 1 μCi of [^{3}H]thymidine. [^{3}H]Thymidine incorporation was determined using a scintillation counter. Data presented are an average of triplicate wells in one of four similar experiments performed with different donors.

Antigen Presentation Assay

To measure the efficiency of presentation of a soluble antigen (tetanus toxoid antigen), 2×10^5 T cells were cultured with 5×10^4 GM-CSF and IL-4 or GM-CSF and IL-13 cultured DC that had been irradiated at 3,000 rad. Cells were cultured in the presence of different concentrations of tetanus toxoid as indicated in 200 μl RPMI-10% FCS in flat-bottom microtiter plates. [^{3}H]Thymidine incorporation was measured after 72 h.

Transduction of DC

DC were grown in GM-CSF and IL-4 or GM-CSF and IL-13 and harvested on days 6–7 as described. DC were resuspended in Aim V medium and plated in 24-well plates at 3×10^5 cells per well in 0.6 ml of medium. Cells were transduced with Ad1.0CMVBgal virus (27) at various multiplicities of infection (MOIs) as indicated for 2 hrs at 37°C in 5% CO_2. After incubation, 0.6 ml of cytokine-containing medium was added to each well for a final concentration of 800 U/ml GM-CSF and 100 ng/ml IL-4 or 800 U/ml GM-CSF and 5 ng/ml IL-13. The cells were returned to culture at 37°C in 5% CO_2 for the indicated number of days.

β-Galactosidase Staining

DC were assayed for β-galactosidase expression 3, 5, and 7 days after transduction with Ad1.0CMVBgal virus. At each time point, cell supernatants were aspirated from each well. Adherent cells were washed once with PBS

and fixed by adding 1 ml per well of X-gal fixative solution (PBS containing 2% formaldehyde, 0.05% glutaraldehyde) and incubated for 5 min at 37°C. Wells were washed two times with PBS; then they were incubated for 3 h at 37°C with 0.5 ml of X-gal staining solution (PBS containing 5 m*M* potassium ferrocyanide, 5 m*M* potassium ferrocyanide, 2 m*M* $MgCl_2$, and 1 mg/ml X-gal). After incubation, wells were washed two times with PBS and left in 1 ml of PBS to take photographs and determine transduction efficiency. Percentages indicate the number of blue cells with DC morphology compared with the total number of DC per high-power field.

RESULTS

PBMC from healthy donors or breast carcinoma and melanoma patients were plated at 6×10^6/ml in T150 flasks in 25 ml of media. After a 2-h adherence step at 37°C, half of the flasks received media containing GM-CSF (800 units/ml) and IL-4 (100 ng/ml) and the other half received media containing GM-CSF (800 U/ml) and IL-13 (5 ng/ml). The flasks were incubated for 6–7 days at 37°C, after which cells were harvested, counted, and phenotyped. The phenotype of the cells generated using IL-13 versus IL-4 was the same for both the healthy donor and patient samples; both populations were $CD3^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD54^+$, $CD58^+$, $CD86^+$, and HLA DR^+ as expected for DC. The purity of the two populations of DC, as determined by flow cytometry analysis, also was not significantly different. Furthermore, the yield of DC (number of DC harvested/total number of input PBMC) was also the same for DC generated using GM-CSF and IL-4 or GM-CSF and IL-13 from both healthy donors and cancer patients (Table 1).

DC have been shown to be potent stimulators in generating a MLR response. We have shown previously that DC generated using GM-CSF and IL-4 were 100-fold more potent at inducing an allogenic MLR response compared with PBMC from the same donor (unpublished results). The ability of DC generated using GM-CSF and IL-13 to induce an MLR response was compared with that of DC generated using GM-CSF and IL-4. In experiments looking at DC generated using both GM-CSF and IL-4 and GM-CSF and IL-13 from four different donors, the results from an MLR were comparable (Fig. 1). We also compared the ability of these two populations of DC to present varying concentrations of tetanus toxoid antigen to autologous T cells. Again the results using DC generated with GM-CSF plus IL-4 or GM-CSF plus IL-13 were comparable (Fig. 2).

In our studies, we have been interested in using DC generated in GM-CSF and IL-4 to present peptide antigens to generate $CD8^+$ CTL specific for peptide antigens. We next evaluated the ability of DC generated using GM-CSF and IL-13 to generate CTL in these protocols. In the first series of experiments, we looked at the HLA-A2 restricted flu specific CTL that were generated from healthy donor $CD8^+$ responders after one or two stimulations using DC generated in GM-CSF and IL-13 compared with those generated in GM-CSF and IL-4. Both DC populations could generate flu-specific CTL in the presence of IL-7, which could kill flu-pulsed T2 cells compared with T2 cells, which were pulsed with an irrelevant peptide (Fig. 3). In several experiments, the cytotoxicity seen using GM-CSF and IL-13 DC was higher than that using GM-CSF and IL-4 DC from the same donor. In addition, the phenotype of the CTL generated from these two sets of DC was comparable; both populations of CTL were >90% $CD3^+8^+4^-$ and $CD56^-$. Con-

TABLE 1. *Comparison of phenotype, purity, and yield of DC cultured in GM-CSF and IL-4 and DC cultured in GM-CSF and IL-13*

	GM-CSF and IL-4 DC						GM-CSF and IL-13 DC					
Donor no.	%CD86	%HLA-DR	%CD14	%CD58	%Purity[a]	%Yield[b]	%CD86	%HLA-DR	%CD14	%CD58	%Purity	%Yield
HD 1	94	97	0	88	37	2.9	96	83	0	21	37	1.6
HD 2	69	69	1	99	68	2.5	57	59	1	99	68	2.5
HD 3	63	90	1	97	39	6.1	66	90	1	97	52	5.3
HD 4	92	94	2	87	26	3.6	75	82	2	98	39	4.2
HD 5	90	97	2	91	57	2.0	90	93	2	83	53	3.8
CP 1	94	95	1	97	77	8	94	95	0	95	72	6.5
CP 2	90	88	6	96	42	8.7	93	92	15	97	46	9.8
CP 3	87	92	12	98	38	3.9	84	87	12	96	38	7.5
p value					0.322	0.496						

DC, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-4, interleukin-4; HD, healthy donor; CP, carcinoma patient.
[a] Percent purity determined by fluorescence activated cell sorter analysis as described in Materials and Methods section.
[b] Percent yield determined by the following formula: (total no. of DC harvested)/(total # of cells plated) × 100.

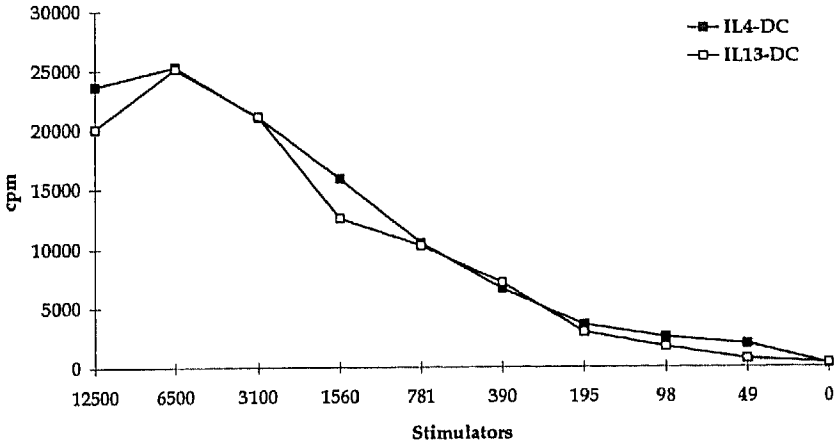

FIG. 1. Mixed lymphocyte response using interleukin-4 (IL-4) versus IL-13 dendritic cells (DC). Peripheral blood mononuclear cells from one donor were divided in two and DC were generated using granulocyte-macrophage colony-stimulating factor (GM-CSF) (800 U/ml) plus IL-4 (100 ng/ml) and using GM-CSF (800 U/ml) plus IL-13 (5 ng/ml). Varying numbers of IL-4 and IL-13 DC were used to stimulate allogeneic T cells as indicated. Proliferation was determined after 5 days of stimulation. Data are representative of one of four experiments.

trol cultures stimulated with unpulsed DC, DC pulsed with an irrelevant peptide, or with IL-7 only showed no specific cytotoxicity.

We next evaluated the ability of the IL-13 DC to generate CTL specific for the MART-1 tumor-associated antigen peptide. We and others have previously demonstrated that MART-1-specific CTL can be generated from both healthy donors and melanoma patients using DC generated in GM-CSF plus IL-4 (Fig. 4, top). DC generated in GM-CSF and IL-13 are also able to generate MART-1 specific CTL from autologous $CD8^+$ cells. Thirty to 40% cytotoxicity could be detected on T2 target cells pulsed with MART-1 peptide compared with background levels seen on T2 cells pulsed with an irrelevant peptide (Fig. 4, bottom). No specific cytotoxicity could be seen when unpulsed DC plus IL-7 or IL-7 only was used to stimulate the cultures. These results are comparable to that seen with IL-4 DC.

Gene-modified DC may have advantages over peptide-pulsed DC for immunotherapy protocols. Therefore, we compared the adenovirus transduction efficiency of GM-CSF plus IL-4 versus GM-CSF plus IL-13-generated DC. DC were prepared as described above and infected on day 6 of culture with Ad1.0CMVBGal virus at various MOI ranging from 25 to 400. DC were assayed for β-galactosidase expression on day 3, 5, and 7 postinfection. DC generated in IL-13 and IL-4 both showed ~60% expression at the highest MOI (Table 2). Expres-

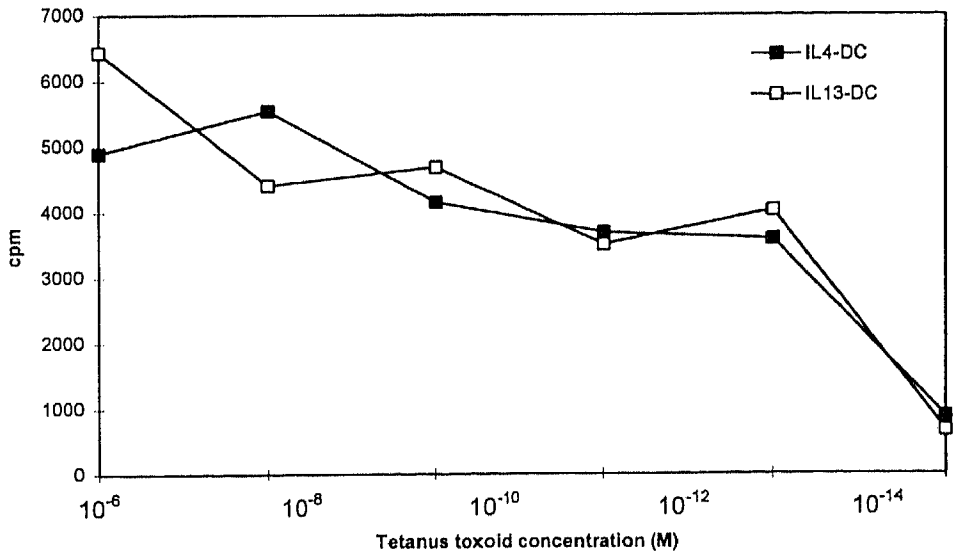

FIG. 2. Tetanus toxoid response using interleukin-4 (IL-4) versus IL-13 dendritic cells (DC). Peripheral blood mononuclear cells from one donor were divided in two and DC were generated using granulocyte-macrophage colony-stimulating factor (GM-CSF) (800 U/ml) plus IL-4 (100 ng/ml) and using GM-CSF (800 U/ml) plus IL-13 (5 ng/ml). DC were used to stimulate autologous T cells in the presence of varying concentrations of tetanus toxoid as indicated. Proliferation was determined after 5 days of stimulation. Data are representative of one of four experiments.

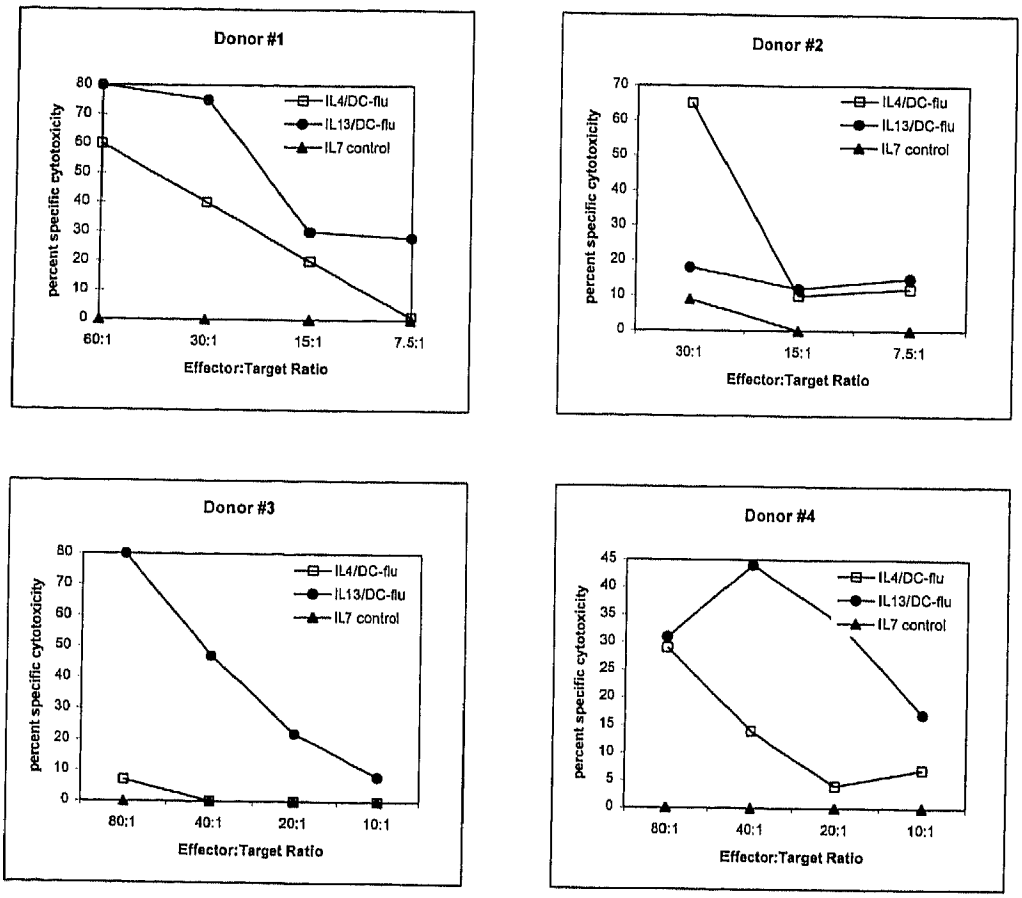

FIG. 3. Generation of a flu peptide response using interleukin-4 (IL-4) versus IL-13 dendritic cells (DC). CD8 responders from four different healthy HLA-A2$^+$ donors were stimulated with autologous flu peptide-pulsed DC that had been generated in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) plus IL-4 or GM-CSF plus IL-13 as described. During stimulation, cells were cultured in 10 ng/ml IL-7. Cytotoxicity was assayed after one or two rounds of stimulation with flu peptide-pulsed DC-IL-7 at the indicated effector:target ratios. The response was assayed on T2 target cells pulsed with flu peptide as well as on T2 cells pulsed with an irrelevant peptide; the response on T2 cells pulsed with the irrelevant peptide was usually at background levels and has been subtracted. Control cultures were cultured in 10 ng/ml IL-7.

sion levels for both DC looked better on days 5 and 7 compared with day 3.

DISCUSSION

An emerging area of cancer immunotherapy includes the use of DC to activate tumor-specific CTL. It has been previously shown that culture of PBMC with GM-CSF and IL-4 induces maturation of a DC population that is able to process and present tumor antigen, generate tumor-specific CTL, and that can protect against and reverse tumor growth in various mouse models of cancer. In our current study we investigated whether IL-13, which has many properties similar to those of IL-4, can substitute for IL-4 in the generation of DC with the potential to be used for tumor immunotherapy.

Our results indicate that PBMC cultured with GM-CSF and IL-13 do induce the differentiation of DC. This conclusion is based on a variety of observations. First, we have shown that when we compared the yield and purity of GM-CSF and IL-4 versus GM-CSF and IL-13 DC isolated from the same donor (either from healthy volunteers or cancer patients), we found comparable results (Table 1). In addition, DC cultured in GM-CSF and IL-4 or GM-CSF and IL-13 both give rise to DC with similar phenotype (CD86$^+$HLA-DR$^+$CD14$^-$CD58$^+$). In a similar study, Piemonti et al. (28) looked at the phenotype of DC generated in GM-CSF and IL-13 and also found that the phenotype of these cells was comparable to those generated in GM-CSF and IL-4. In their study, they looked at many additional cell surface markers including CD1a, ICAM1, CD18, CD11a, CD11b, and CD11c.

We also looked at the function of the two populations of cells by comparing their ability to process and present antigen to CD4$^+$ T cells and to generate antigen-specific

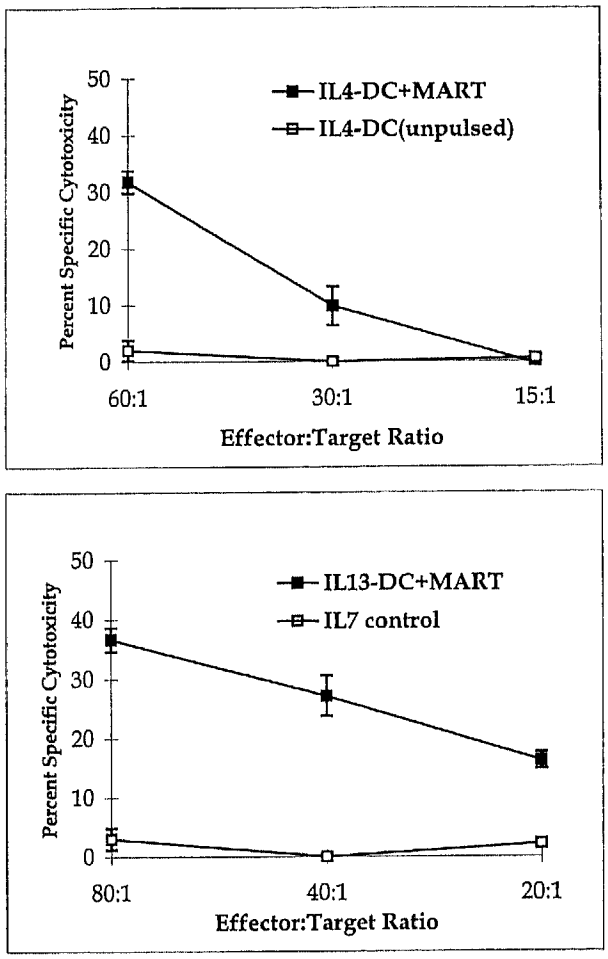

FIG. 4. Generation of a MART peptide response using interleukin-4 (IL-4) versus IL-13 dendritic cells (DC). CD8 responders were stimulated with MART pulsed DC that had been generated in the presence of granulocyte-macrophage colony-stimulating factor plus IL-4 **(top)** or GM-CSF plus IL-13 **(bottom).** Stimulators were cultured in 10 ng/ml IL-7. Cytotoxicity was assayed after two rounds of stimulation with MART peptide-pulsed DC-IL-7 at the indicated effector:target ratios. The response is given on T2 target cells pulsed with MART peptide; the background response on T2 cells pulsed with an irrelevant peptide has been subtracted. Control cultures were cultured with unpulsed DC in the presence of 10 ng/ml IL-7 or IL-7 only. Previous experiments indicate that these controls both give background levels of cytotoxicity.

$CD8^+$ CTL for potential tumor immunotherapy applications. The two populations of DC isolated from the same individual using GM-CSF and IL-4 or GM-CSF and IL-13 were both able to stimulate allogeneic T cells in an MLR (Fig. 1) and to process and present tetanus toxoid antigen (Fig. 2). When we compared the ability of the GM-CSF and IL-13 DC versus the GM-CSF and IL-4 DC isolated from the same donor to generate antigen-specific CTL against the HLA-A2 restricted flu peptide, we found that both DC populations generated CTL that killed targets in an antigen-specific manner (Fig. 3).

TABLE 2. *Comparison of infection efficiency of Ad1.0CMVBgal in interleukin-13-dendritic cells (IL-13-DC and IL-4-DC)*

	Day 3		Day 5		Day 7	
MOI	IL-4-DC	IL-13-DC	IL-4-DC	IL-13-DC	IL-4-DC	IL-13-DC
400	50–60[a]	30–40	60–70	50–60	50–70	50–60
200	30–40	25–30	50–60	50	40–50	40–50
100	10–20	10–15	30	30	30–40	30–40
50	10	5	15–20	15	20	20–30
25	5	1–5	10	10	5–10	15–20
0	1	0	5	5	0	1–5

MOI, multiplicity of infection.
[a] Percent of cells staining blue after X-gal stain.

Similar results were found using the tumor antigen MART-1 (Fig. 4). The CTL generated from these studies had the same phenotype regardless of whether the DC were cultured in GM-CSF and IL-4 or GM-CSF and IL-13.

The ability of DC to express the entire tumor antigen gene may have a number of advantages for tumor immunotherapy compared with peptide-based strategies. These include the expression of multiple and undefined epitopes and the fact that these epitopes will be presented in the context of any MHC. When a comparison of gene transfer methods was made in human DC, adenoviral vectors were shown to be promising for transgene delivery (29). Adenovirally infected DC have recently been shown to stimulate human virus–specific CTL in vitro (30) and to elicit protection from a MART-1-expressing tumor line in animal models. When we compared DC generated in GM-CSF and IL-4 and DC generated in GM-CSF and IL-13 for the ability to be transduced by adenovirus, we determined that both gave similar results.

When the function of GM-CSF and IL-4-differentiated DC isolated from breast carcinoma patients was compared with that of mature peripheral blood DC, it was determined that although the latter had considerably lower than normal ability to stimulate allogeneic T cells and present soluble antigen, the GM-CSF and IL-4-stimulated DC were able to elicit an almost normal level of immune response (31). DC generated in GM-CSF and IL-4 are currently being assessed for tumor immunotherapy in several clinical trials. Our results suggest that DC generated in GM-CSF and IL-13 may show similar effects in tumor immunotherapy and gene therapy protocols using DC strategies.

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